CHAPTER 15

Comparison of Effects of Two Pesticides on Soil Organisms in Laboratory Tests, Microcosms, and in the Field

J. Römbke, Th. Knacker, B. Förster, and A. Marcinkowski

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ABSTRACT

The objective of the study presented here was to compare ecotoxicological data obtained from the field with data obtained from laboratory test systems and microcosms. The intention of the comparison was to find out whether microcosms can be considered as a useful tool to obtain ecologically relevant data when investigating the ecotoxicological effects of chemical stressors. Two pesticides, the insecticide E 605 (active ingredient: Parathion) and the herbicide Ustinex (active ingredients: Amitrole and Diuron) were used as chemical stressors. Each pesticide was applied in two concentration levels on a grassland field site and on soil-core microcosms extracted from the grassland. In addition, the pesticides were tested by using laboratory test systems. Test parameters were the abundance and biomass of earthworms (Lumbricidae) and Enchytraeidae. With regard to the parameter observed, the chemical applied and the organizational levels investigated, each oligachaete family reacted to each of the pesticides in a specific manner. However, the ability to mimic effects of pesticides observed under field conditions was much better when using microcosms compared to results obtained from laboratory test systems.

I. INTRODUCTION

National and international legislations on plant protection require data to evaluate the effects of agrochemicals to terrestrial ecosystems. Usually, this evaluation is based on results derived from laboratory test systems, though these tests are not representative for ecosystems.[1] However, field tests which can represent ecosystems are difficult to reproduce, costly, and time consuming. An alternative approach is the use of microcosms or model ecosystems.[2]

The objective of the study presented here was to compare ecotoxicological data obtained from the field with data obtained from laboratory test systems and microcosms which have been developed at Battelle.[3] The intention of the comparison was to find out whether or not microcosms can be considered as a useful tool to obtain ecologically relevant data when investigating the ecotoxicological effects of chemical stressors. As such stressors the herbicide Ustinex, containing the active ingredients Amitrole and Diuron, and the insecticide E 605, containing the active ingredient Parathion, were selected. The abundance and biomass of enchytraeids and earthworms were chosen to quantify the possible ecotoxicological effects on all three differently complex organizational levels: laboratory, microcosms, and field. The Oligochaeta were chosen as representative and important members of the soil fauna which are typical for many soils of the temperate zones.[4]

II. MATERIALS AND METHODS

The laboratory tests with earthworms were performed according to the OECD Guideline No. 207 using the compost worm *Eisenia fetida*.[5] For enchytraeids, a laboratory test system developed at Battelle was performed using the species *Enchytraeus albidus*.[6] In both tests the parameter mortality and the effects on biomass, reproduction, and changes of behavior were determined.

The soil cores (= microcosms) with a depth of 60 cm and a diameter of 18 cm were extracted from the field without affecting the layering of the soil. The soil cores, encased in high-density polyethylene tubes, were transported to the greenhouse and placed in temperature-controlled wooden containers. A detailed description of the extraction and storage of these microcosms is given by Knacker et al.[3]

The field study was performed on the same grassland site from where the soil cores were extracted. The grassland was a *Arrhenathereum elatioris* association with rows of apple trees at the boundaries located approximately 10 km north of Frankfurt a. M. The mean annual temperature was 9.8°C, the average precipitation was 648 mm. The site was divided into four treated plots and a control plot, each covering an area of 25 m^2. The site was within a distance of approximately 10 m to the plot where the soil cores were extracted. The soil was a brown soil on loess. The control plot and the soil core plot showed a pH value of 5.8 and an organic matter content of 2.4%, whereas the plots treated with chemicals revealed higher pH values of 6.5 to 7.7 and organic matter contents of 2.7 to 3.1%.

This variation of the physicochemical properties of the soil was accompanied by differences in the enchytraeid species composition which were not observed in the microcosms. With regard to abundance, biomass, and species composition, the oligochaete populations were within the normal range found for other European grasslands.[7] The dominant enchytraeid species, *Achaeta microcosmi*, was described from this site for the first time.[8]

Each month and for each treatment and the control, one earthworm and one enchytraeid sample was collected from a single microcosm. The destructive sampling was conducted by using a split-core extractor for the enchytraeids samples. The remaining soil of the microcosm was then hand sorted to collect the earthworms. For microcosms there were no replicate samples per sampling date.

In the field, three replicate earthworm and enchytraeid samples were taken per plot. For earthworms the method of hand sorting, followed by a Formol treatment (0.3% formalin solution applied three times after intervals of 10 min) was used. For enchytraeids, a split-core extractor was used to collect the samples. The enchytraeids were extracted from the soil by using the wet-funnel method.

Table 1. Amount of Test Chemicals Applied on the Microcosms and the Field (A) milliliters of E 605 and grams of Ustinex per Hectare (B) milligrams of E 605 and milligrams of Ustinex per kg Dry Weight of Soil

	E 605		Ustinex	
Plot marking	E1	E5	U1	U5
A	610	3000	1000	5000
B	0.6	3.0	10	50

Samples were taken starting in April 1989, i.e., 3 months prior to application, until September 1989, and additionally in May 1990 1 year after application.

The insecticide E 605 forte (active ingredient: Parathion) and the herbicide Ustinex (active ingredients: Amitrole and Diuron), which are commonly used in apple orchards, were selected as test chemicals. In a laboratory study using sandy loam comparable to the soil of the grassland described above, Parathion showed a half-life of about 4 weeks.[9] Amitrole persists in soil for 2 to 4 weeks and diuron for 4 to 8 months.[10]

E 605 and Ustinex were applied to the field plots and microcosms in the highest recommended application rate and in a fivefold higher concentration once in June 1989 (Table 1). These concentrations were transferred to mg/kg using the equation: 1000 g/ha equals 1 mg/kg. In the field the tests chemicals were applied using a parcel sprayer. In the greenhouse the chemicals were applied on top of the microcosms using micropipettes. For each of the four treatments and the control, one field plot and eight microcosms, respectively, were used. E 605 caused no visible effect on the vegetation. Ustinex, however, eliminated nearly all plants and caused an increased soil moisture both in the field and in the microcosms (Figure 1).[11]

III. RESULTS AND DISCUSSION

A. Population Dynamics in the Microcosms and the Field

One purpose of this study was to find out whether or not the type of microcosm described in this report could be a useful tool to predict the ecotoxicological effects of chemicals on Oligochaeta living in natural environments. To answer this question it was necessary to demonstrate the similarity of the population dynamics in the field and in the microcosms.

Using the biomass as an example, it is shown in Figure 2 that in fact the population dynamics of enchytraeids in the field and microcosms were comparable, although in the microcosms the abundance and biomass were higher than in the field. It is assumed that this was caused by the high soil moisture of the soil cores in the greenhouse where additional watering was required to avoid harmful stress for the plants. Despite the fact that *F. galba* was always

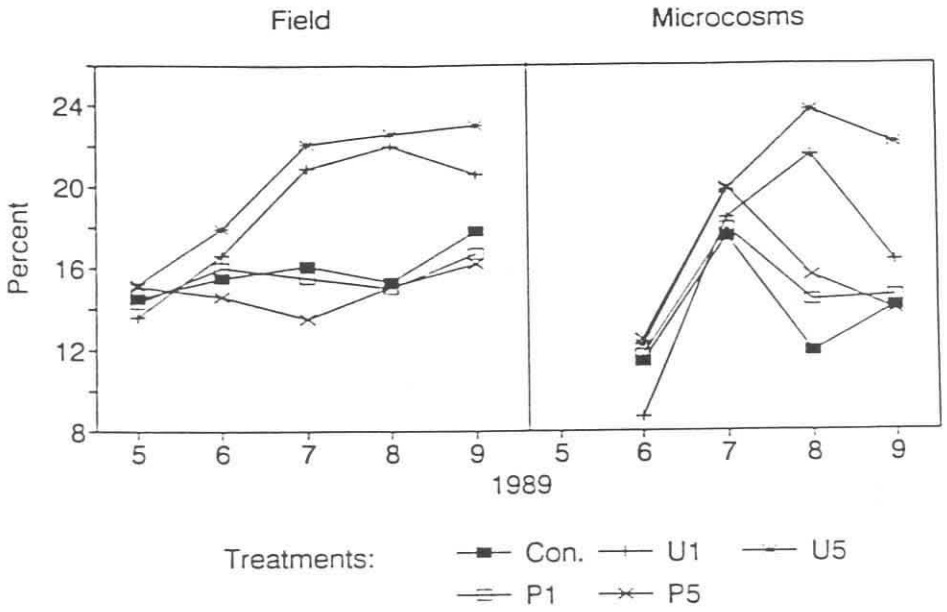

FIGURE 1. Soil moisture in the field and in the microcosms.

the dominant species when considering biomass, changes in the species composition occurred. Especially, the amount of *A. microcosmi* was much higher in the microcosms compared to the field. However, the ratio of juvenile to adult worms as well as the vertical distribution of the enchytraeids were not modified in the microcosms.[12]

In the soil cores the number of earthworms was slightly increased compared to the field, while the biomass decreased (Figure 3). The typical annual fluctuation of the population with a minimum in summer and a maximum in autumn

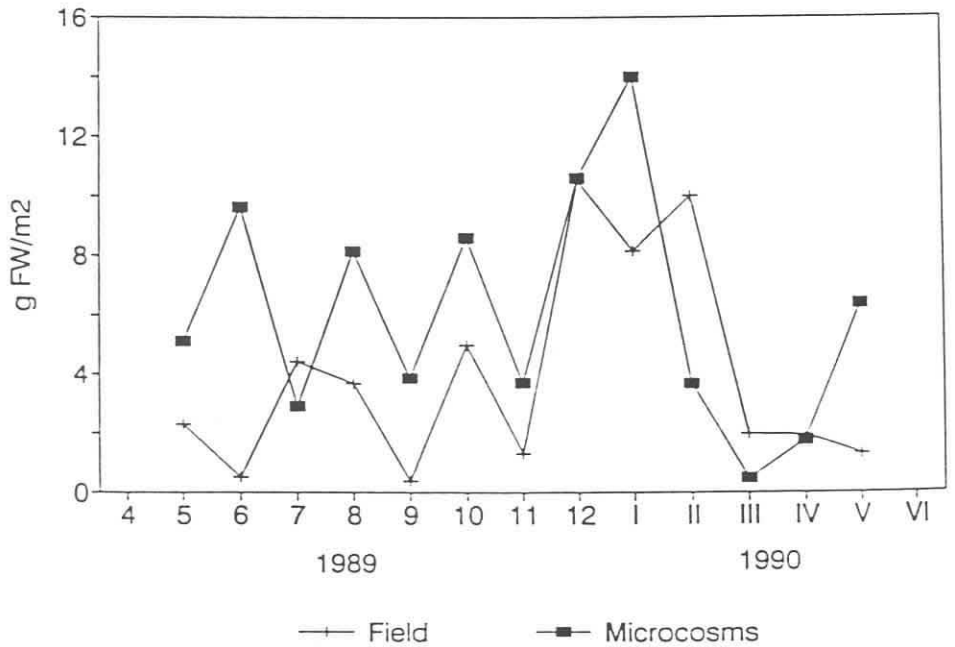

FIGURE 2. Biomass of the Enchytraeidae in the control plot of the field and in the untreated microcosms.

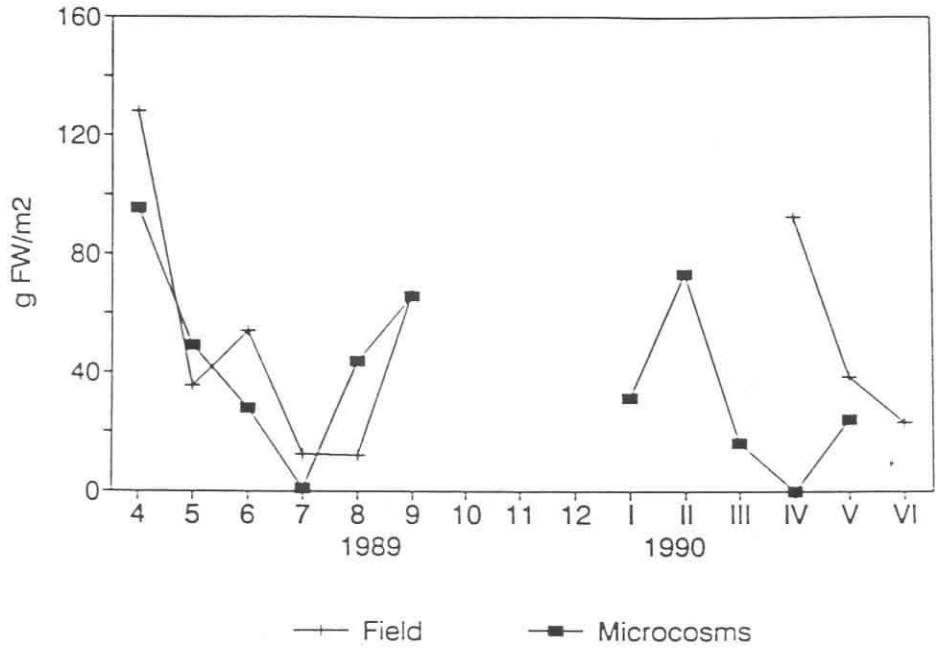

FIGURE 3. Biomass of the earthworms in the control plot of the field and in the untreated microcosms.

and spring was measured in the field as well as in microcosms. However, in comparison to the field the number of earthworm species decreased in the microcosms from 12 to 10. Especially, the large species *Lumbricus terrestris* and *Aporrectodea longa*, dominant in the field plots, were hardly found in the soil cores. Moreover, the individual weight of the worms was reduced in the microcosms by approximately a factor of two. The difference between microcosm and field was thought to be caused by the size of the soil cores and the limited amount of food for earthworms in the soil cores.

The standard variation of the replicate samples of enchytraeids in the field was on an average ± 66%, which can be expected to be similar in the microcosms. Considering the variability of these data, which are in the same range as in other investigations,[13] the population dynamics of the worms in the microcosms showed excellent correlation with the development of the oligochaete cenosis in the field. As a result it can be stated that the specific microcosms design maintained abiotic and biotic conditions which allowed us to investigate members of the soil fauna for at least one vegetation period.

Based on these findings, it was expected that the ecotoxicological effects caused by the pesticides in the field and the microcosms were similar. First, however, the results obtained when applying the test materials on laboratory test systems are shown.

B. Laboratory Tests With Pesticides

In the laboratory tests, Ustinex caused neither effects on earthworms nor on enchytraeids up to a concentration of 1000 mg/kg. In the literature, however,

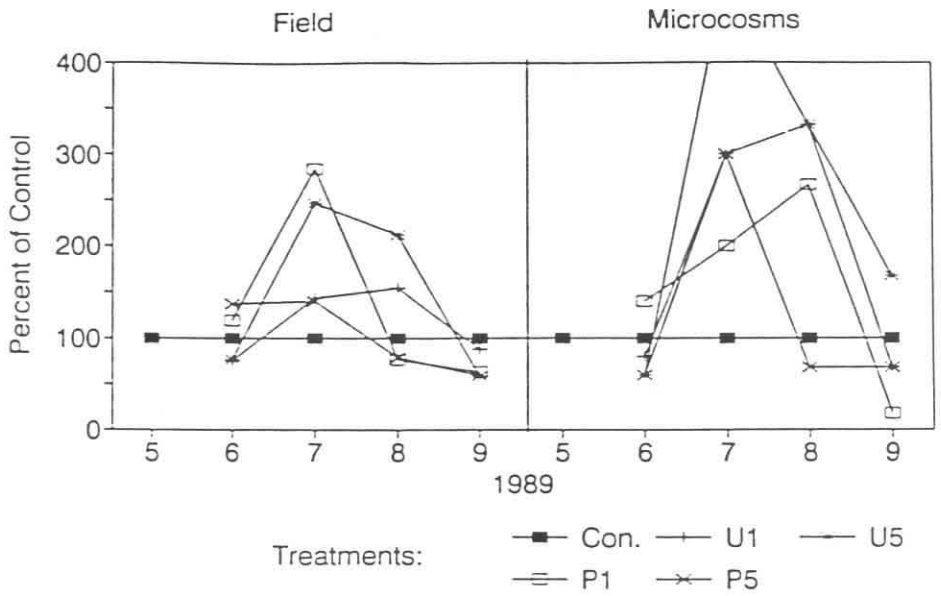

FIGURE 4. Effects of the test chemicals on the abundance of earthworms in the field and in the microcosms.

effects of the active ingredient Amitrole were reported: the biomass of the earthworm *A. caliginosa* was reduced under laboratory conditions at a concentration of 100 mg/kg.[14] The effects of Ustinex on Enchytraeidae were investigated in this study for the first time.

When applying E 605, LC_{50} values of 135 mg/kg for the earthworm *Eisenia fetida* and 124 mg/kg for the enchytraeid *Enchytraeus albidus* were determined. Moreover, effects on the biomass at approximately 100 mg/kg, on the reproduction and on the behavior at 10 mg/kg were detected. These findings were in agreement with those reported from other authors who measured an LC_{50} value of 34 mg/kg for *L. terrestris* and an LC_{50} value ranging between 100 and 1000 mg/kg for *Eisenia fetida*.[15] For the latter species, effects on biomass and reproduction were found at concentrations of 10 to 32 mg/kg.

Bethge-Beilfuss and Westheide[16] investigated the effects of Parathion on enchytraeids by using agar as a test substrate, which can be considered as an aqueous medium for enchytraeids. Dependent on the species selected and the test conditions chosen, the effects of Parathion were found to range between 0.21 and 21 mg/kg in soil.

C. Microcosm and Field Studies With Pesticides

Since all microcosms were extracted from within a small area of the grassland, the fluctuations of the soil characteristics in the microcosms were narrow. Consequently, the variation of the abundance for both the earthworms and enchytraeids (Figures 4 and 5) in the microcosms was small when comparing the data obtained from all treatments at the beginning of the investigation. In all treated soil cores, increased earthworm abundance and biomass were measured 1 month after application of the chemicals. This increase was lower in the

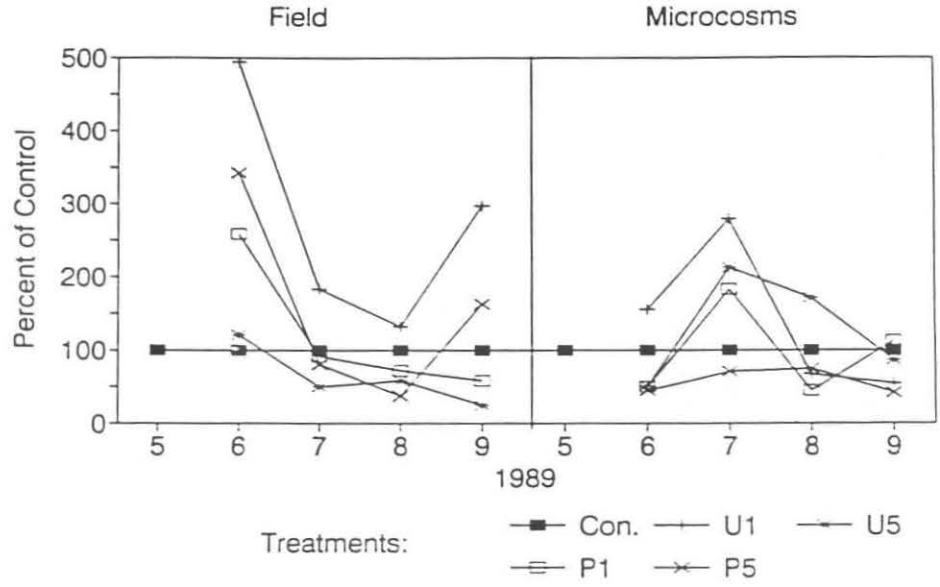

FIGURE 5. Effects of the test chemicals on the abundance of Enchytraeidae in the field and in the microcosms.

microcosms treated with E 605 than in those treated with Ustinex. In all soil cores a return to control levels or below was observed after 3 months (Figure 4).

Despite the variability of the soil properties in the field, Figure 4 shows that there were no obvious differences between the earthworm cenoses of the grassland plots before applying the chemicals. Immediately after application the number and biomass of the Lumbricidae increased for 2 months and returned to the level determined for the control plot. On the plot treated with the high E 605 concentration, the timely limited increase of the abundance was less pronounced than on the other plots.

The effects of the chemicals on the enchytraeids in the microcosms were comparable to those measured for the earthworms. Only in the microcosms treated with the high concentration of E 605, the number and biomass decreased (Figure 5).

When comparing the grassland plots before applying the pesticides the enchytraeid cenoses were rather different (Figure 5). In particular, their abundance on all treated plots was higher than on the control plot. Despite the difficulty of comparing the treated plots with the control, it seemed that there were no significant changes in number and biomass during the study period.

For Table 2, the single values derived from the microcosms on a monthly basis during the investigation period from June to September 1989 were averaged to calculate the mean abundance and biomass as well as the total species number for enchytraeids and earthworms. In Table 3, the triplicate values derived from the field were exhibited as in Table 2.

The abundance of earthworms was enhanced when microcosms were treated with Ustinex. The biomass increased when the recommended application rate was used for Ustinex and E 605, but decreased at the fivefold higher E 605

Table 2. Effects of the Test Chemicals Ustinex (U1 and U5) and E 605 (E1 and E5) on Earthworms and Enchytraeidae in the Microcosms

	U1	U5	E1	E5	Control
Earthworms					
Number of species	4	4	5	5	5
Mean abundance/m²	247	388	211	141	176
Mean biomass g/m²	47	39	54	22	34
Enchytraeidae					
Number of species	14	17	15	14	15
Mean abundance/m²	44,200	36,400	25,300	18,800	34,100
Mean biomass g/m²	7.7	11.7	7.1	2.8	6.1

Note: Average values based on four single samples taken between June and September 1989.

concentration (Table 2). In soil cores treated with Ustinex, juveniles of the genus *Octolasion* were most abundant while in all other microcosms juveniles of the genus *Aporrectodea* were dominant.[12]

Compared to the control the abundance of the Enchytraeidae was increased in the soil cores treated with the low Ustinex concentration, while the biomass was enhanced only in those microcosms treated with the high Ustinex concentration. The high E 605 concentration reduced the number and biomass of the enchytraeids (Table 2). Although *Achaeta microcosmi* was the dominant species in all microcosms concerning number and *F. galba* concerning biomass, changes in the dominance spectrum occurred in all treated soil cores except those treated with the low Ustinex concentration.[12]

In Table 3 it is shown that on average the biomass of the earthworms on the field plot treated with the low Ustinex concentration was increased, whereas no changes occurred on the other plots. The individual weight of most lumbricid species was enhanced on the Ustinex plots and reduced on the E 605 plots compared to the control. The weight increase of individual organisms was at least partly due to the enhanced amount of dead organic matter, which was available as food on the soil surface after application of the herbicide Ustinex,

Table 3. Effects of the Test Chemicals Ustinex (U1 and U5) and E 605 (E1 and E5) on Earthworms and Enchytraeidae in the Field

	U1	U5	E1	E5	Control
Earthworms					
Number of species	10	10	9	10	10
Mean abundance/m²	101	115	108	86	94
Mean biomass g/m²	48	40	32	29	36
Enchytraeidae					
Number of species	18	15	15	17	13
Mean abundance/m²	22,300	5,900	10,400	11,400	9,700
Mean biomass g/m²	5.2	0.9	2.6	2.6	2.2

Note: Average values based on four triplicate samples taken between June and September 1989.

whereas the reduction of biomass observed at the end of the investigation period (Figure 4) was probably caused by food shortages, since the herbicidal effect lasted for approximately half a year and did not allow any growth of plants. The population dynamics observed on the field plots treated with E 605 (Table 3, Figure 4) could be due to the reduction of epigean earthworm predators like carabid or staphylinid beetles, which were certainly affected by the insecticide.[17]

For Enchytraeidae, the abundance and biomass were increased on the plots treated with the low Ustinex and reduced on the plots treated with the high Ustinex concentration (Table 3). Based on average values covering the entire study period, the treatment with E 605 caused hardly any effect on the abundance and biomass of enchytraeids in the field (Table 3). However, on all treated plots the number of species was slightly higher than on the control plot. Additionally, both the high and low concentration of E 605 altered the ratio of juvenile to adult individuals.[12]

Other field investigations with the two pesticides are scarce (data from microcosms are not available). For example, Van Rhee[18] found small alterations of the behavior and reproduction of the earthworm *A. caliginosa* at much lower concentrations of Ustinex (1 to 5 kg/ha, corresponding to 1 to 5 mg/kg) than in laboratory tests.

A review of Haque and Pflugmacher[19] on the effects of Parathion to earthworms in the field revealed that some authors found effects,[20] while others, even at high application rates of 4.5 to 9 l/ha, could not detect any effect on the worms.[21] Unfortunately, the data basis of these studies is limited and in some cases appropriate methods have not been used.

A reduction of the number of enchytraeids, particularly juveniles, was measured in a loamy and sandy soil treated with Parathion under field conditions.[20] However, due to an inappropriate extraction method used by Weber,[20] which leads to underestimated numbers of enchytraeids, the results are difficult to compare with the results obtained in this study.

IV. CONCLUSIONS

In general, the Enchytraeidae were easier to collect and seemed to react slightly more sensitive to the agrochemicals than the earthworms. On the other hand, the determination to the species level and the evaluation of the biomass was more time consuming for enchytraeids than for earthworms.

To characterize ecotoxicological effects it seems that the number and biomass of the worms are sensitive parameters. The evaluation of the ecotoxicological effects on single species has not been completed yet. However, there are several indications that in both oligochaete families single species react specifically towards chemical stress.

Summarizing the results, it is important to remember that in comparison with the results of the laboratory tests the concentrations of the test chemicals

applied in the microcosms and in the field were within the range where acute effects were not expected to occur.

In the microcosms, the high concentration of E 605 caused negative effects on the biomass of earthworms and enchytraeids, whereas the treatment with Ustinex enhanced the number and biomass of the Oligochaeta. In the field, the low Ustinex concentration produced effects corresponding to the results obtained from microcosms, while at the high concentration the number and biomass of enchytraeids were decreased and the number and biomass of earthworms remained unchanged.

The ability to mimic effects of pesticides observed under field conditions was much better when using microcosms compared to results obtained from laboratory test systems. However, the decrease of the enchytraeid population on the field plot treated with the high Ustinex concentration was not reflected by results derived from microcosms.

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